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Case Report: Molecular Diagnosis of Subcutaneous *Spirometra erinaceieuropaei* Sparganosis in a Japanese Immigrant

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Abstract. We report a case of subcutaneous sparganosis in a 68-year-old female Japanese immigrant in Germany. The patient complained of a painless erythema caudal of the umbilicus with a palpable subcutaneous cherry-sized lump. Polymerase chain reaction on formalin-fixed parasite tissue identified *Spirometra erinaceieuropaei* as the causative agent; the proliferative form of sparganosis, which is caused by the branching and disseminating *Sparganum proliferum*, could, thus, be excluded. From the excised sparganum, an immunofluorescence test was established and revealed an antibody response directed against the parasite's tegument. Histological key features of the plerocercoid that facilitate diagnosis with different stains are presented.

INTRODUCTION

Sparganosis is a neglected parasitic disease caused by the plerocercoid stage (sparganum; third-stage larva) of the pseudophyllidean tapeworm genus *Spirometra*. Human sparganosis is most often seen in East Asia, particularly in China, Japan, Korea, Thailand, and Vietnam.[1] Various *Spirometra* species may infect humans, and in many cases, the exact species has not been determined. Sparganosis in the Old World is most likely caused by *Sp. erinaceieuropaei* (*S. mansoni*), whereas *S. mansonoides* occurs in the New World. Infections with the different species show no evident differences in clinical presentation, except for disease caused by the more pathogenic *Sparganum proliferum*. The latter is a provisionally termed larval cestode for which the adult strobilar stage is so far unknown.[2] This parasite shows excessive branching, budding, and dissemination in the human host, and infection may take a lethal course. Most cases of infection with this species are reported from Japan.[2] Humans become infected with *Spirometra* species by eating the raw or undercooked meat of mainly amphibians and reptiles, which act as second intermediate or paratenic hosts and contain the plerocercoids. Because the parasite is not very host-restricted at the plerocercoid stage, many vertebrate species, such as chickens or fish, can also become intermediate hosts and act as a source for infections of both humans (dead-end intermediate hosts) or cats and dogs, the natural final hosts. Other modes of transmission include the application of raw plerocercoid-infested flesh from intermediate hosts on conjunctiva, mucosa, or open wounds and the subsequent migration of the larvae into the human body. Drinking of unsanitized water containing copepods infected with procercoids, the second-stage larvae, can also lead to the development of sparganosis. The spargana may invade the brain, eyes, viscera, and subcutis and cause serious illness.[3]

The present report describes a case of subcutaneous sparganosis caused by *S. erinaceieuropaei* in a Japanese woman who immigrated to Germany 42 years ago. Diagnosis was established by histopathological investigations and confirmed by polymerase chain reaction (PCR). Sequencing of the amplicon allowed an exact species diagnosis and thus, the exclusion of an infection with *S. proliferum*.

CASE REPORT

In November of 2010, a 68-year-old female Japanese immigrant was seen in the Department of Plastic Surgery and Hand Surgery at the Klinikum rechts der Isar in Munich, and she had a circumscribed erythema measuring 1 cm in diameter distal to the umbilicus. On palpation, a 1-cm^3 painless and non-itching subcutaneous lump was discovered. The swelling had developed over 6 months without any migratory sensation. No other skin lesions were present. In July of 2010, an invasive ductal breast cancer had been diagnosed, and the patient had been treated with breast-conserving therapy and radiotherapy until October (cumulative dose of 60 Gy). The patient had immigrated to Germany 42 years before from Japan. The patient had been born and raised in Tokyo. Ever since that time, she had been visiting relatives in Japan several times a year and had consumed local dishes. The consumption of raw snake meat was denied. The patient had also traveled to China 3 years before. Imported canned food from Japan had regularly been consumed in Germany.

Laboratory investigations and ultrasonographic examination were not performed, because the patient decided to undergo surgery rapidly to clarify the etiology of the tumorous lesion. The lump was excised under local anesthesia without any complications.

During surgery, several 4- to 5-cm-long flattened pseudosegmented helminthic structures were extracted from the subcutaneous lump. The parasite material was immediately fixed in formalin and embedded in paraffin for additional histological examination. The excised parasite tissue did not show any strobilar structures (Figure 1). Morphological and histological analysis of the helminths showed aspects typical of cestodes (e.g., calcareous corpuscles) in a spongiform stroma surrounded by an aspinous tegument. As a characteristic feature of pseudophyllidean cestode larvae (plerocercoids or spargana), the anterior regions showed invaginations and no proper scoleces (Figure 2A). Figure 2 shows the histological key features in detail, such as the distribution of muscle fibers and gland cells of the sparganum.

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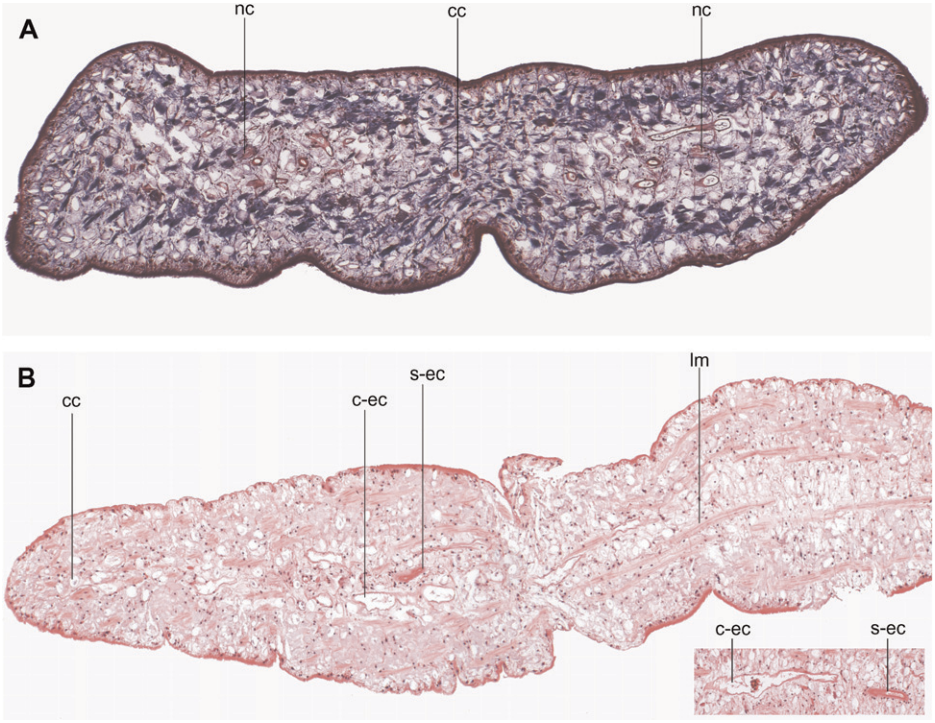

FIGURE 1. Longitudinal section through the parasite. (**A**) The histoarchitecture of the parasite is reminiscent of a cortical and medullary organization. The longitudinal and traversing dorsoventral musculature encompasses a region where the tubular canals of the excretory system concentrate and the ramification of the terminal ductules of the nephridial system is considerably developed. Lillie's trichrome stain. Original magnification: 10×. (**B**) Plerocercoid with main as well as secondary branches of the excretory system and numerous longitudinal muscle fibers, both extending throughout the entire parasite. Areas of narrowing, ridges, and inward folds run the full length. Hematoxylin and eosin stain. Original magnification: 10×. (**Inset**) Collecting excretory canal with luminal content and tributary excretory canal. The latter has thicker and more deeply staining walls, a circular muscle layer, and surrounding long-shafted mural cells. Hematoxylin and eosin stain. Original magnification: 10×. cc = calcareous corpuscle; c-ec = collecting canal of the excretory system (supposedly); lm = longitudinal muscle; nc = nerve cord; s-ec = secondary excretory canal.

To determine the exact parasite species and exclude an infection with *S. proliferum*, DNA was extracted from fixed helminths and subjected to a cestode-specific mitochondrial 12S rRNA gene and cytochrome c oxidase subunit 1 (*cox1*) PCR using the newly designed primers 12S*Taenia*FF (5′-CAC AGT GCC AGC ATC YGC GGT-3′) and 12S*Taenia*RR (5′-GAG GGT GAC GGG CGG TGT GTA C-3′) and previously published and frequently used *cox1* primers.[4] Sequence analysis of the 440- and 425-bp amplicons of the 12S rRNA gene and *cox1* PCR showed homologies of 100%, respectively, to *S. erinaceieuropaei* sequences in GenBank (accession numbers AB374543.1 [Japanese isolate], GU946438 [Chinese isolate], AF096237.2 [Korean isolate], and AF096238.2 [Korean isolate]). Histological sections of the parasite were also used to set up an immunofluorescence test (IFT). In brief, slides were deparaffinized and incubated with different concentrations of the patient's serum. After incubation with a fluorescein isothiocyanate (FITC) -labeled anti-human secondary antibody (Sifin, Berlin, Germany), a tegumental signal was detected at a concentration of up to 1:100 (Figure 3). A similar signal was seen with serum of a patient with *S. proliferum* sparganosis at this low concentration (data not shown). When the IFT was incubated with sera from patients with echinococcosis and cysticercosis, unspecific staining of the parenchyma, but not the tegument, was seen at a dilution of up to 1:100. A serum sample from a patient without any known helminth infection was negative at the same dilution. The sparganosis patient's serum showed no antibodies against *Taenia solium* in an enzyme-linked immunosorbent assay (ELISA; DRG, Marburg, Germany) and immunoblot (Immunetics, Brussels, Belgium). The patient did not receive any anthelminthic chemotherapy, and a 1-year follow-up period was uneventful.

DISCUSSION

More than 1,400 human cases of sparganosis have been reported globally, including travel-related and migration-associated cases.[5] Only a few autochthonous cases of sparganosis have been reported from Europe.[6] *S. erinaceieuropaei* is the parasite most often implicated in the Old World. The vast majority of cases occur in Asia, presumably because of the local eating habits. Sparganosis is an emerging food-borne parasitic disease in the People's Republic of China, with approximately 1,000 cases between 1927 and 2007.[7] The consumption, in particular, of raw frog and snake meat from local food markets, where wild-caught animals are sold, is a risk factor. Necropsies of wild-caught frogs and tadpoles revealed prevalence of 20–27% and 12% in China, respectively, with an infection intensity of up to 15 spargana per frog.[7,8] Because snakes prey on amphibia, high prevalence rates of 30–48% have been documented in snakes from food markets and the wild in China.[9,10] The parasite is also present in Japan, and it was found in cats (final host) with a prevalence of 8.3%.[11]

The incubation period is not well-defined, and the parasite may live up to 20 years in the human body. In subcutaneous sparganosis, an incubation period between 1 day and several months has been estimated.[12–14] In the case presented here,

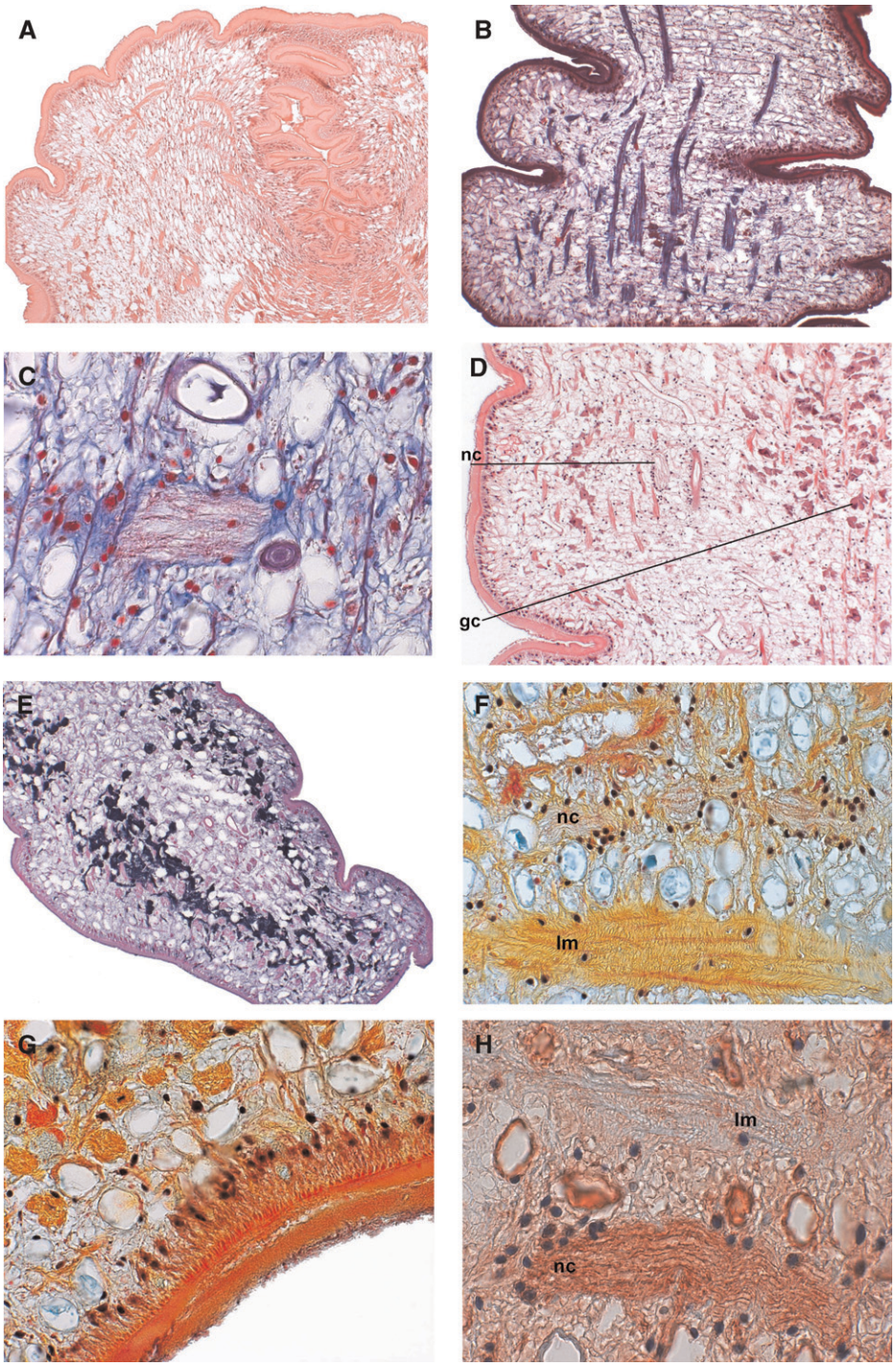

FIGURE 2. Close-up views of the excised plerocercoid. (**A**) Tortuous appearance of an apical indentation in the anterior region of a developing acaudate bothrio-plerocercoid with pseudosegmented, furrowed appearance. Hematoxylin and eosin stain. Original magnification: 10×. (**B**) Proximal of the indentation, the parasite's tegument is noticeably thickened. Lillie's trichrome stain. Original magnification: 10×. (**C**) Nerve cord (central structure) and calcareous corpuscle (concentric structure) between reticular connective tissue fibrils. Heidenhain's azan stain. Original magnification: 40×. (**D**) Nucleated bodies of irregular but mostly piriform shape were seen within an area situated beneath the apical indentation. These structures are filled by spherical granules and probably correspond to gland cells (gc). nc = nerve cord. Hematoxylin and eosin stain. Original magnification: 10×. (**E**) Parts of the presumptive gland apparatus in the distal part of the plerocercoid (dark nucleated and granular-filled structures). Gomori's chrome-alum-hematoxylin-phloxin stain. Original magnification: 10×. (**F**) Detail of two of the somatic organ systems. Nerve cord and bundle of longitudinal smooth muscle fibers with contractile myofilaments. The remnants of the concentric lamellae of calcareous corpuscles are stained with Alcian blue. lm = longitudinal muscle. Movat's pentachrome stain. Original magnification: 20×. (**G**) Detail of the metacestode's syncytial tegument. In the distal cytoplasm, microtriches, as an enlargement of the surface, form a brush border. The outer anucleate zone with subjacent basal membrane complex is followed by the nucleated inner zone (proximal cytoplasm) with subtegumental cells (cytons or perikarya) and columnar internuncial processes connecting the tegumentary cytons to the distal syncytium. Movat's pentachrome stain. Original magnification: 40×. (**H**) S-100 immunohistochemical staining of one of the nerve cords showed it to be continuous throughout the entire organism as one single strand. The clear ovoid spaces are caused by dissolved calcareous corpuscles. Original magnification: 40×.

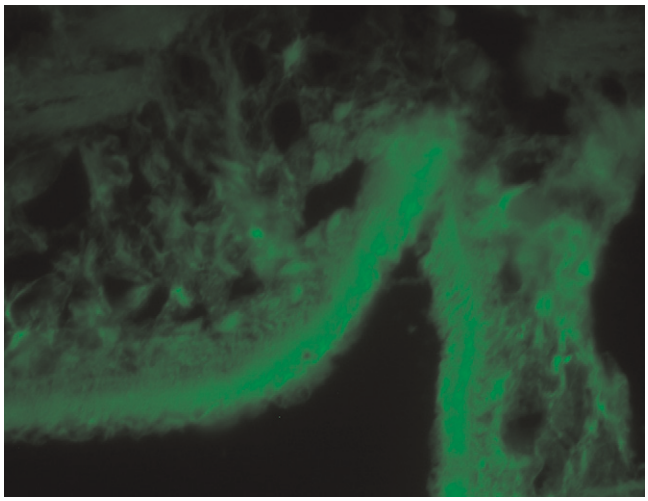

FIGURE 3. Immunofluorescence test with a section through the formalin-fixed paraffin-embedded sparganum from the patient's lesion. An increased fluorescence signal on the parasite's tegument is visible at a serum dilution of 1:100. Original magnification: 63×.

the time and the source of infection remain unclear. The consumption of reptile or amphibian meat was denied. However, the consumption of possibly undercooked chicken or wild boar meat as a source cannot be excluded. The molecular sequence of the isolated sparganum did not provide clues to the locality where the infection was possibly acquired, because there were no sequence differences to Japanese, Chinese, or Korean isolates. Most likely, however, the infection was acquired in Japan, because the patient frequently visited her home country. The plerocercoid may migrate to any part of the body, but preferred infection sites are the breast, abdomen, scrotum, legs, and central nervous system. The most serious complication is neurosparganosis, which develops in 3.2% of patients.[15] In a recent analysis of parasitic encephalopathies, cerebral sparganosis was seen in 15.4% of cases in China.[16] Pain is often caused when the parasite, which reaches lengths of several centimeters (sometimes up to 18–50 cm),[17,18] moves in the subcutis[1] or dies. In the case presented, the localization of the helminth was typical, but pain was not reported. It remains unclear whether a possible immunosuppression caused by the breast cancer led to a more rapid development or migration of the parasite or fewer symptoms because of a comparatively undisturbed development.

In some cases of subcutaneous sparganosis, imaging techniques have provided useful information before surgery. Magnetic resonance imaging showed low-signal, bundle-like structures and high-signal structures on T1- and T2-weighted images, respectively.[19] Ultrasonography showed hyperechoic, hypoechoic, and anechoic lesions with internal serpinginous tubular structures and elongated hyperechoic masses with hypoechoic signals in the central portion.[20,21] Thus, imaging techniques may provide diagnostic hints, but these methods do not allow a definitive diagnosis and leave a broad range of differential diagnoses to be considered, including infections with other metacestodes and neoplasias. The diagnosis of sparganosis, therefore, requires removal and examination of the helminth. However, an exact species diagnosis based on morphology can only be achieved by feeding the plerocercoid to a definitive host followed by an examination of the adult strobilar stage, which is hardly practicable.[22,23] Metacestodes of different genera, such as the elongated strobilicercus of *T. taeniaeformis*, bizarre cysticercoids of *Hymenolepis nana*, and tetrathyridia of Old World *Mesocestoides lineatus* and New World *M. variabilis* (all Cyclophyllidea), have to be considered in the differential diagnosis after the helminth has been recovered.

In the present case, PCR analysis followed by sequencing of the amplicons clearly identified the species responsible as *S. erinaceieuropaei*. The determination of the exact species is of epidemiological interest, but far more importantly, it allowed the exclusion of the more pathogenic *S. proliferum*. Serological data from the 1990s suggested a close relationship between *S. proliferum* and *S. erinaceieuropaei*,[24] and earlier studies postulated that *S. proliferum* might be a virus-infected or abnormally differentiated *S. erinaceieuropaei*.[25,26] However, recent genetic inferences from mitochondrial cox1 genes unambiguously showed that *S. proliferum* is a distinct species from *S. erinaceieuropaei* in the same order of the Pseudophyllidea.[2,27] For prognostic reasons, we argue for molecular investigations in addition to histopathology in patients with sparganosis to accurately discriminate *Spirometra* species from the disseminating *S. proliferum*.

Serological tests may be valuable for the diagnosis in cases where it is impossible to resect the parasite for examination. Using two ELISAs with excretory/secretory products and crude antigen extracts of *S. mansoni*, a study conducted with 20 sparganosis patients showed sensitivities of 100% but specificities of 97% and 72%, respectively.[28] Cross-reactions were seen in sera from patients infected with various platyhelminths as expected. In contrast, higher specificities were described with an ELISA that used recombinant parasite cysteine proteinase,[29] and a two-dimensional immunoblot analysis with specifically reacting spots has been developed.[30] The detection of declining antibody titers to spargana after removal of the helminth to confirm a complete resection has also been shown.[31] A signal on the parasite's tegument was described in an IFT based on frozen sparganum sections at a serum dilution of 1:400 before surgery in a recent case.[31] We here established an IFT based on formalin-fixed paraffin-embedded histological sections of the excised parasite, showing a similar tegumental signal when incubated with the patient's serum. Unfortunately, only one serum sample was available, which was drawn a few weeks after surgery, and it exhibited a low titer of 1:100. A titer of 1:100 must be considered very low and may even be caused by unspecific fluorescence. Keeping in mind the high titers that were seen in previous reports[31] it may be assumed that the titer had already declined because of the parasite resection. Considering the possibility of a suppressed immune system and the limited symptoms of the patient as well as the lack of degeneration of the parasite, it seems also possible that the patient had not developed much higher antibody titers anyway before surgery. This hypothesis is supported by the finding of a similarly low tegumental fluorescence titer in the serum before surgery of a different (and immunocompetent) patient suffering from a confirmed infection with a non-degenerated *S. proliferum*. The IFT is, thus, not suitable for a species diagnosis and has limitations because of cross-reactions and/or unspecificity with patchy parenchymal signals in other helminth infections; however, this method might be useful to show a declining antibody titer after removal of the parasite in patients with an initially high titer.

Surgical excision remains the definitive treatment modality, but antiparasitic chemotherapy with praziquantel, mebendazole, or topical ethanol has been tried with some success.[19,32] The surgical approach without anthelminthic therapy was successful in our case. Regular follow-up examinations for 1 year did not show any additional symptoms of infection. Especially if an infection with *S. proliferum* has not been excluded, a patient should be closely monitored. In the case of a definitely diagnosed or suspected infection with *S. proliferum*, additional anthelminthic therapy should be considered, even if the benefits of such chemotherapy are still to be elucidated.

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